

## A Simple Method for Suppressing Dispersion-Mode Contributions in NMR Spectra: The "Pseudo Echo"

High-resolution NMR spectra are usually displayed in the absorption mode, since the dispersion-mode signal has long tails which interfere with neighboring lines. However, many two-dimensional Fourier transform experiments generate responses in which absorption ( $v$  mode) and dispersion ( $u$  mode) are inextricably mixed—the so-called "phase twist" lineshape (1, 2). Sections through the center of such a two-dimensional response, if taken parallel to the  $F_1$  or  $F_2$  axes, show a pure absorption mode profile; all other sections contain an admixture of dispersion mode. When two such lines overlap there are undesirable interference effects, but a far more serious problem arises when homonuclear two-dimensional  $J$  spectra are projected in a direction at  $45^\circ$  to the positive  $F_1$  and  $F_2$  axes in order to remove homonuclear spin-spin splittings (3). All intensities in the projected spectrum vanish since the positive and negative parts of the phase twist response cancel identically (4). "Decoupled" proton spectra are therefore obtained by projection of absolute value mode signals,  $(u^2 + v^2)^{1/2}$ , an unsatisfactory solution because of the long tails associated with this lineshape (5).

This communication suggests a general method of eliminating dispersion-mode components from a free-induction decay. The idea derives from the observation that when suitably phased, the two halves of a spin echo contain absorption-mode components in the same phase but dispersion components in antiphase. If  $T_2 \gg T_2^*$ , it is therefore feasible to eliminate the antisymmetric component either by Fourier transformation of the complete echo (6) or by transformation of the two halves of the echo separately, followed by addition of the resulting spectra (7). Phase-sensitive two-dimensional spectra in the pure absorption mode have been obtained by this method (6).

This property is not restricted to spin echoes. Any time-domain signal with an envelope which decays in a symmetrical fashion on each side of the midpoint of the time axis has a frequency-domain spectrum in which antisymmetric components are eliminated. Conventional free-induction signals are easily converted into such "pseudo echoes" by multiplication by a suitable shaping function. Suppose that the free-induction signal is acquired for a total period of  $2T$  sec and decays exponentially according to  $S = S_0 \exp(-t/T_2^*)$ . The first step is to cancel this instrumental decay by multiplying by  $\exp(+t/T_2^*)$  and then shape the resulting signal so that it decays symmetrically about  $t = T$ , the midpoint of the time domain. See Fig. 1. In many cases a Gaussian is a convenient choice for the form of this decay function, giving a Gaussian lineshape in the transformed spectrum. A suitable shaping function would then be

$$f(t) = \exp(+t/T_2^*) \exp[-(T - t)^2/G^2], \quad [1]$$

where the full linewidth in the frequency domain is  $5/(3\pi G)$  Hz.

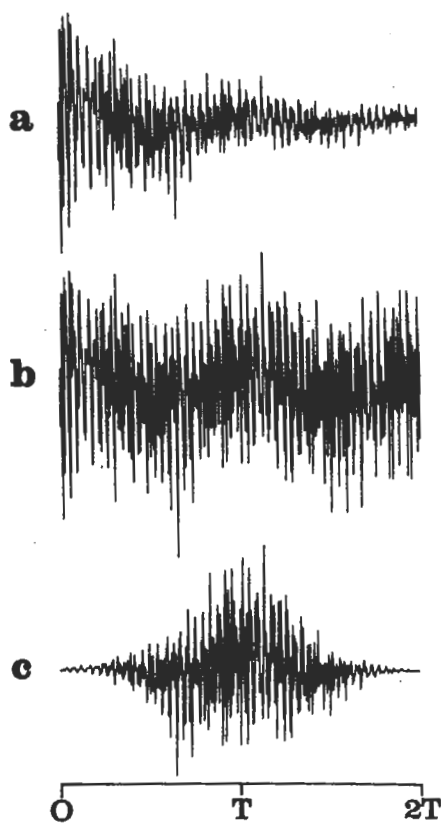


FIG. 1. (a) An experimental free-induction signal with an exponentially decaying envelope. (b) The same signal multiplied by  $\exp(+t/T_2^*)$ . (c) A "pseudo echo" obtained by reshaping (b) to give a Gaussian envelope symmetrical about  $t = T$ .

Thus a pseudo echo differs from a true echo in that the various frequency components are not in phase at the center,  $t = T$ , but at  $t = 0$ , which introduces a large frequency-dependent phase shift into the transformed spectrum. A further complication is that while both real and imaginary parts of the Fourier transform of such a pseudo echo show an "absorption-mode" lineshape, the relative magnitudes of the real and imaginary components depend on the phase of the signal at the midpoint of the pseudo echo (6). These problems are readily circumvented by calculating the square root of the sum of the squares of the real and imaginary components of the transform, since, once the antisymmetric  $u$  mode has been canceled, this is simply the modulus of the  $v$ -mode signal.

The reshaping of a free-induction decay into a pseudo echo incurs a severe penalty in sensitivity. Early parts of the free-induction signal, where the signal-to-noise ratio is high, contribute very little to the pseudo echo, the sensitivity being primarily determined by the signal-to-noise in the central region near  $t = T$ . Sensitivity is degraded by a factor of the order of  $\exp(+T/T_2^*)$  and this rapidly becomes more unfavorable if higher resolution is required, since this implies a

longer acquisition time for the technique.

In many practical applications, consideration of the experimental free-induction decay, thus centering the pseudo echo, and the antisymmetric components, that the weighting function impose effective symmetry, wiggles in the frequency domain should be set so that  $G$  is a function of time. Digital resolution of the pseudo echo.

The method of pseudo echo is used in a two-dimensional proton  $J$  spectroscopy. An example was provided by (1SR) decan-2,5-dione. Ideally carried out in both time dimensions, enhancement was employed in the  $t_2$  dimension. This very good multiplet resolution was used in the  $t_1$  dimension.

In the other dimension the half of a spin echo, acquired

$$f(t_2)$$

At first sight it seems strange but in practice it is not possible if  $t_1$  is less than  $t_2$ .

The spectra are illustrated. In the conventional spectrum these multiplets, in this direction, they coalesce at shift frequencies (Fig. 2, center). In the present example there is a significant rise (in the AB case) giving rise to a significant intensity (8). The

When spin-spin relaxation there are small unresolved lines in the projected spectrum as a consequence of variations in relative intensities. Each peak should represent a spin-spin relaxation. The OH line, which is broad

longer acquisition time  $2T$ . This appears to be the most severe limitation on the technique.

In many practical applications this loss of sensitivity may be the overriding consideration. If the signal-to-noise ratio is too low at the midpoint of the experimental free-induction decay, it may be necessary to choose a shorter value of  $T$ , thus centering the pseudo echo at an earlier point in the free-induction signal. Antisymmetric components are still canceled to a good approximation provided that the weighting function used to shape the pseudo echo is severe enough to impose effective symmetry. In order to ensure this, and to avoid sinc function wiggles in the frequency domain, the Gaussian weighting parameter  $G$  of Eq. [1] should be set so that  $G \leq T/3^{1/2}$ . The shorter the setting of  $G$ , the lower the resolution. Digital resolution may be improved by zero-filling on one or both sides of the pseudo echo.

The method of pseudo echoes has been applied to the problem of projecting two-dimensional proton  $J$  spectra. These were obtained at 200 MHz on a Varian XL-200 spectrometer. An example of a proton spectrum with many long-range couplings was provided by (1SR, 4RS, 7RS, 9SR, 10RS)-9-hydroxytricyclo[5,2,1,0<sup>4,10</sup>]decan-2,5-dione. Ideally the conversion to a symmetrical pseudo echo would be carried out in both time dimensions,  $t_1$  and  $t_2$ ; in practice, conventional resolution enhancement was employed in  $t_1$  and the reshaping into a pseudo echo occurred in the  $t_2$  dimension. This prevented any severe loss in sensitivity while achieving very good multiplet resolution. A standard Lorentzian-Gaussian transformation was used in the  $t_1$  dimension,

$$f(t_1) = \exp[t_1/0.7 - t_1^2/(1.7)^2]. \quad [2]$$

In the other dimension the transient signal  $S(t_2)$  was obtained from the second half of a spin echo, acquired for 0.64 sec. It was reshaped by the function

$$f(t_2) = \exp[t_2/0.3 - (t_2 - 0.32)^2/(0.2)^2]. \quad [3]$$

At first sight it seems strange to convert half of a true spin echo into a pseudo echo, but in practice it is not possible to acquire the first half of the echo because in many cases  $t_1$  is less than  $t_2$ .

The spectra are illustrated in Fig. 2. Note the extensive overlap of proton multiplets in the conventional spectrum (Fig. 2, top). In the two-dimensional spectrum these multiplets lie on 45° diagonals, and when a projection is made in this direction, they coalesce to give a spectrum consisting of lines at the chemical shift frequencies (Fig. 2, center). This presupposes first-order spin-spin coupling. In the present example there are two instances of strongly coupled pairs of protons, giving rise (in the AB case) to lines at the mean chemical shift  $(1/2)(\nu_A + \nu_B)$  with significant intensity (8). These "spurious" lines are marked "SC" in the diagram.

When spin-spin relaxation times vary significantly from line to line, or when there are small unresolved long-range couplings, intensity anomalies appear in the projected spectrum as a consequence of the displacement of the time origin. These variations in relative intensity are apparent in the central trace of Fig. 2, since each peak should represent a single proton, at least for the weakly coupled sites. The OH line, which is broadened by exchange, shows the effect of a short spin-

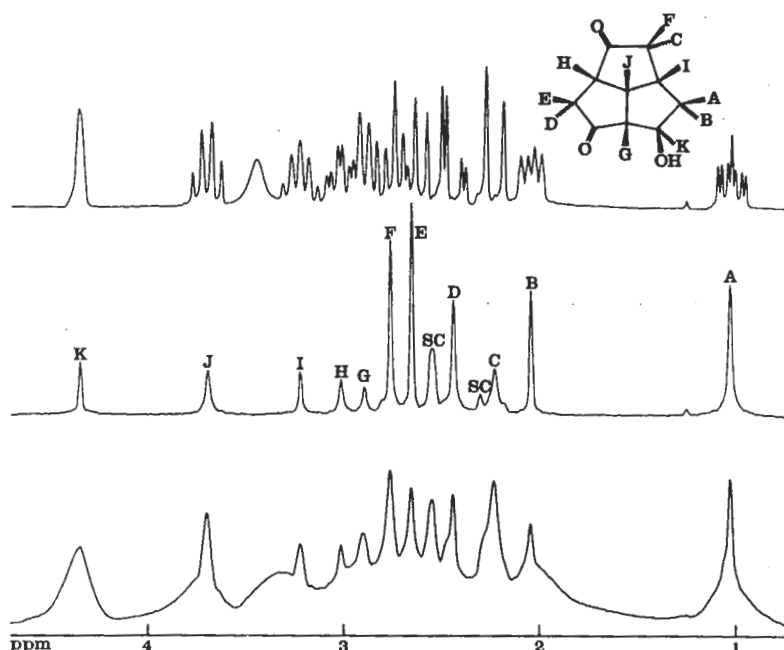


FIG. 2. The conventional 200-MHz proton spectrum (top trace) of the molecule sketched in the inset. The central trace shows the 45° projection of the two-dimensional  $J$  spectrum, with pseudo echoes used in the  $t_2$  domain to suppress dispersion-mode contributions. Lines marked "SC" arise as artifacts of the strong proton coupling. The lowest trace is a 45° projection of a two-dimensional spectrum in the absolute-value mode obtained from the same data as those of the central trace, but without reshaping of the time-domain signals.

spin relaxation time, for it makes very little contribution to the pseudo echo and hence is not apparent in the central trace of Fig. 2.

A comparison may be made with the 45° projection of the two-dimensional spectrum in the usual absolute-value mode (Fig. 2, lowest trace). This involved no reshaping of the time-domain signals into pseudo echoes and no resolution enhancement. Strong tails extend on each side of the lines. Particularly broad profiles are observed for projections of lines with partially resolved multiplet structure, for example line K is split by at least four small couplings.

If two absorption-mode resonances are only partially resolved, the signals are additive in the region of overlap, and the error involved in measuring the separation is small and calculable. If, however, there are dispersion-mode components in the tails, as in the usual absolute-value display, considerable distortion can occur because the dispersion signals have opposite phases in the overlap region. Hence the importance of aiming for complete cancellation of dispersion contributions. The pseudo-echo technique emphasizes that this limiting case is achieved if the envelope of the time-domain signal is symmetrical about its midpoint. One very important application is the 45° projection of two-dimensional proton  $J$  spectra to give spectra without proton-proton splittings (3). Hitherto, this problem has been approached by shaping the time-domain signal in such a way as to diminish

the contribution from the dispersion enhancement function difference (10). Li Gaussian transformation of the enhancement function of

In the usual application of the method so as to cancel the exponential decay, the parameter  $b$  would determine the time to exaggerate the rising exponential. If the acquisition goes through a maximum of the acquisition period, the function is equivalent to the envelope of half-width ( $b^{-1}$ ) is essential for the cancellation. The function accomplishes this. In situations where sensitivity is low, free-induction decays into the noise provides a simple method of obtaining two-dimensional spectra.

One important practical problem arises when the lines of interest are close to the solvent peak, or a diagonal. Multiple-trace display and weak lines will be poorly defined. This problem has been achieved by so that the  $|v|$ -mode signal

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1. G. BODENHAUSEN, R. FREEMAN, *J. Am. Chem. Soc.*, **99**, 133 (1977).
2. P. BACHMANN, W. P. AUE, L. J. W. P. AUE, J. KARHAN, AND I. K. NAGAYAMA, P. BACHMANN, W. BREMSER, H. D. W. HILL, A. BAX, A. F. MEHLKOPF, AND R. FREEMAN, S. P. KEMPEL, G. BODENHAUSEN, R. FREEMAN, A. DEMARCO AND K. WÜTHRICH, I. D. CAMPBELL, C. M. DOBSON (1973).
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the contribution from the tails of the absolute-value lineshape. Typical resolution enhancement functions that have been used are the sine bell (9) and convolution difference (10). Lindon and Ferrige (11, 12) advocate the Lorentzian-Gaussian transformation for reshaping the absolute-value signal using a resolution enhancement function of the form

$$f(t) = \exp(+at/T - bt^2/T^2). \quad [4]$$

In the usual application of this transformation, the parameter  $a$  would be chosen so as to cancel the exponential decay of the free-induction signal, while the parameter  $b$  would determine the extent of the imposed Gaussian decay. It is possible to exaggerate the rising exponential term so that  $a \gg 1$  and the overall shaping function goes through a maximum. This maximum can be centered at  $T/2$ , the midpoint of the acquisition period, by setting  $a = b$ , and under these conditions the shaping function is equivalent to the "pseudo echo" defined above, having a pure Gaussian envelope of half-width  $(b^{-1} \ln 2)^{1/2}$ . Note that the choice of a Gaussian shape is not essential for the cancellation of dispersive components—any *symmetric* shaping function accomplishes this.

In situations where sensitivity is not an important consideration, reshaping of free-induction decays into pseudo echoes in order to display the pure  $v$ -mode signal provides a simple method of avoiding the difficulties of phasing one- or two-dimensional spectra.

One important practical difficulty with two-dimensional NMR spectra arises when the lines of interest lie close to some much stronger line, for example, a solvent peak, or a diagonal peak of a Jeener spectrum (13–15). In both the multiple-trace display and the intensity contour plot there is a danger that such weak lines will be poorly characterized or lost altogether. Considerable improvement has been achieved by conversion of the transient signal into a pseudo echo so that the  $|v|$ -mode signal could be displayed (16).

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#### REFERENCES

1. G. BODENHAUSEN, R. FREEMAN, R. NIEDERMEYER, AND D. L. TURNER, *J. Magn. Reson.* **26**, 133 (1977).
2. P. BACHMANN, W. P. AUE, L. MÜLLER, AND R. R. ERNST, *J. Magn. Reson.* **28**, 29 (1977).
3. W. P. AUE, J. KARHAN, AND R. R. ERNST, *J. Chem. Phys.* **64**, 4226 (1976).
4. K. NAGAYAMA, P. BACHMANN, K. WÜTHRICH, AND R. R. ERNST, *J. Magn. Reson.* **31**, 133 (1978).
5. W. BREMSER, H. D. W. HILL, AND R. FREEMAN, *Messtechnik* **79**, 14 (1971).
6. A. BAX, A. F. MEHLKOPF, AND J. SMIDT, *J. Magn. Reson.* **35**, 373 (1979); **40**, 213 (1980).
7. R. FREEMAN, S. P. KEMPEL, AND M. H. LEVITT, *J. Magn. Reson.* **34**, 663 (1979).
8. G. BODENHAUSEN, R. FREEMAN, G. A. MORRIS, AND D. L. TURNER, *J. Magn. Reson.* **31**, 75 (1978).
9. A. DEMARCO AND K. WÜTHRICH, *J. Magn. Reson.* **24**, 201 (1976).
10. I. D. CAMPBELL, C. M. DOBSON, R. J. P. WILLIAMS, AND A. V. XAVIER, *J. Magn. Reson.* **11**, 172 (1973).
11. J. C. LINDON AND A. G. FERRIGE, *J. Magn. Reson.* **36**, 277 (1979).

12. J. C. LINDON AND A. G. FERRIGE, *Progr. NMR Spectrosc.* **14**, 27 (1980).
13. J. JEENER, Ampère International Summer School, Basko Polje, Yugoslavia, 1971.
14. W. P. AUE, E. BARTHOLDI, AND R. R. ERNST, *J. Chem. Phys.* **64**, 2229 (1976).
15. A. BAX, R. FREEMAN, AND G. A. MORRIS, *J. Magn. Reson.* **42**, 164 (1981).
16. A. BAX, to be published.

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## Double Resonance in H

Since the correlation of (1, 2) has been extended to h along two distinct avenues. two species I and S (usually assignment problems, and inaccessible to conventional the heteronuclear couplings splittings are suppressed in coupling and refocusing tech approach, it may be of inter than on chemical shifts. In p for indirect detection of hor actions) by transferring protc phorus-31 (15-17). In this ma tion-dependent proton-protc proton spectrum.

The basic technique for he two 90° proton pulses, separ 90° phosphorus-31 pulse with magnetization is restricted to a the heteronuclear coupling n coordinates in the two-dimens both homo- and heteronuclea the proton and phosphorus tra signals arise for regressive con proton and phosphorus transit transfer to occur (17). The pr accidental cancellations, partic of the same magnitude of  $J_{HI}$  deceptively simple spectra) it intensities associated with the tively, cancellations may be remove frequency degeneracie

In addition to cancellations served by indirect detection is c AKMX system consisting of typical cases, only one hetero result, only the magnetization o The M region of the proton s splittings due to  $J_{AM}$  and  $J_{KM}$ . coupling constants in most cas ways: if the A (or K) proton reg